

Appl. No. 10/089,534  
Amtd. dated October 26, 2005  
Reply to Office Action of July 26, 2005

Docket No. 58009-010600

### AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application:

**Claim 1-12 (canceled)**

**Claim 13 (currently amended)** A method of forming tissue on a bio-membrane including using crystalline beta-form fibroin to allow for the survival, the proliferation and the differentiation of specialized tissue cells of the human body, comprising:

a. seeding a plurality of human tissue cells on a bio-membrane, the bio-membrane ~~including~~ crystalline beta-form fibroin being produced by:

dissolving degummed silk in a solution of lithium bromide in water at a temperature higher than room temperature and at a standard pressure so as to obtain a dissolved solution;

filtering the dissolved solution through a porous ceramic filter;

diluting the dissolved solution with distilled water;

dialyzing the diluted solution using a dialysis membrane with a 3500 molecular weight cut-off so as to obtain a dialyzed solution;

permitting the dialyzed solution to evaporate in polystyrene containers as to obtain a bio-membrane; and

immersing the bio-membrane in a solution of methanol and water to make it crystalline and insoluble in water;

b. permitting the plurality of human tissue cells to proliferate;

c. permitting the plurality of human cells to differentiate; and

d. forming tissue on the bio-membrane.

**Claim 14 (previously presented)** The method of claim 13, wherein the plurality of human tissue cells is a plurality of skin cells, a plurality of liver cells, a plurality of mesothelial cells, a plurality of astrocytes, a plurality of human skeleton osteoblasts, a plurality of tenocytes, a plurality of human tendon fibroblasts, a plurality of chondrocytes, a plurality of cells isolated

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from cartilaginous tissues, a plurality of endothelial cells of blood vessels, a plurality of steroid-secreting cells of the adrenal cortex, a plurality of smooth muscle cells of the *tunica muscularis* of the intestine and blood vessels, a plurality of squamous epithelial cells of the oral cavity, a plurality of squamous epithelial cells of the conjunctiva/cornea or a plurality of human pre-adipocytes of the white adipose connective tissue.

**Claim 15 (withdrawn)** A method of production of a substrate, comprising:

- dissolving degummed silk in a solution of lithium bromide in water at a temperature higher than room temperature and at a standard pressure so as to obtain a dissolved solution;
- filtering the dissolved solution through a porous ceramic filter;
- diluting the dissolved solution with distilled water;
- dialyzing the diluted solution using a membrane with a 3500 molecular weight cut-off so as to obtain a dialyzed solution;
- permitting the dialyzed solution to evaporate in polystyrene containers as to obtain a membrane; and
- immersing the membrane in a solution of methanol and water to make it crystalline and insoluble in water.

**Claim 16 (previously presented)** The method of claim 13, wherein the plurality of human tissue cells originate from one human body.

**Claim 17 (previously presented)** The method of claim 13, wherein the plurality of human tissue cells originate from a plurality of human bodies.

**Claim 18 (previously presented)** The method of claim 13, further comprising:

- providing glucose to the plurality of human tissue cells; and
- permitting the plurality of human tissue cells to consume the glucose and secrete lactic acid.

**Claim 19 (previously presented)** The method of claim 13, wherein the plurality of human tissue cells includes fibroblasts, and further comprising allowing the fibroblasts to secrete extracellular matrix components and precursors to collagen fibers.

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**Claim 20 (previously presented)** The method of claim 13, wherein the plurality of human tissue cells are keratinocytes, and further comprising permitting the keratinocytes to form epithelium.

**Claim 21 (currently amended)** The method of claim 13, wherein the plurality of human tissue cells includes pre-irradiated fibroblasts and keratinocytes, and further comprising allowing the cells to proliferate and function ~~with normal cytological features~~ without an occurrence of diffpoptosis

**Claim 22 (previously presented)** The method according to claim 13, wherein said fibroin is secreted by the *Bombyx mori* silkworm.

**Claim 23 (currently amended)** The method according to claim 13, wherein the bio-membrane ~~includes~~ comprises a polymer of natural origin.

**Claim 24 (previously presented)** The method according to claim 13, wherein the bio-membrane includes a polymer of synthetic origin.

**Claim 25 (previously presented)** The method according to claim 13, wherein the fibroin in the bio-membrane is present in a quantity varying from 20% to 80% in weight.

**Claim 26 (withdrawn)** A method of production of a substrate, comprising:

dissolving crystalline beta form fibroin in a solution of lithium bromide in water at a temperature higher than room temperature and at a standard pressure so as to obtain a dissolved solution;

filtering the dissolved solution through a porous ceramic filter;

diluting the dissolved solution with distilled water;

dialyzing the diluted solution using a membrane with a 3500 molecular weight cut-off so as to obtain a dialyzed solution;

permitting the dialyzed solution to evaporate in polystyrene containers as to obtain a membrane; and

**Claim 27 (new)** A method of forming tissue on a membrane including crystalline beta-form

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fibroin to allow for the survival, the proliferation and the differentiation of specialized tissue cells of the human body, comprising:

- a. seeding a plurality of human tissue cells on a membrane, the membrane being produced by:

dissolving crystalline beta-form fibroin in a solution of lithium bromide in water at a temperature higher than room temperature and at a standard pressure so as to obtain a dissolved solution;

filtering the dissolved solution through a porous ceramic filter;

diluting the dissolved solution with distilled water;

dialyzing the diluted solution using a dialysis membrane with a 3500 molecular weight cut-off so as to obtain a dialyzed solution;

permitting the dialyzed solution to evaporate in polystyrene containers as to obtain a membrane; and

immersing the membrane in a solution of methanol and water to make it crystalline and insoluble in water;

- b. permitting the plurality of human tissue cells to proliferate;  
c. permitting the plurality of human cells to differentiate; and  
d. forming tissue on the membrane.